SOME OBSERVATIONS ON THE IMMUNOCHEMISTRY OF DEXTRANS

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Early studies on the antigenic properties of dextran produced by *Leuconostoc mesenteroides* were conducted with rabbits. Zozaya (1) reported that nitrogen-free dextran was nonantigenic when injected into rabbits but could be rendered antigenic by adsorption upon colloidal carriers such as collodion or alumina. However, FitzGerald (2) could not confirm these results. FitzGerald found that nitrogen-free dextran was incapable of producing antibodies, but preparations containing small amounts of nitrogen stimulated precipitin formation in rabbits. In later serologic studies, Evans and co-workers (3) concluded that dextran was a haptene. They expressed the view that the preparation used by Zozaya was not nitrogen free as claimed. Studies on the antigenicity of clinical dextrans have shown them to be nonantigenic in the guinea pig (4–6) and dog (4).

Zozaya (7) observed that dextran cross-reacted serologically with antisera of some of the pneumococci, certain of the Salmonella group, and with some strains of *Streptococcus viridans*.

Sugg and Hehre (8), using rabbit antisera produced by immunization with *Leuconostoc mesenteroides* organisms grown in sucrose broth, observed precipitin reactions with dextran or with sterile filtrates of sucrose broth cultures. Leuconostoc organisms cultured on glucose broth neither stimulated the production of dextran-reactive antibodies in rabbits, nor absorbed dextran-reactive antibodies from sera as did organisms cultured on sucrose. Sugg, Hehre and associates (8–17) established that dextrans gave serologic cross reactions with the sera of rabbits immunized with Type 2, Type 20, and in some instances with Type 12 pneumococci. These workers (18–20) also demonstrated the capacity of native and partially hydrolyzed dextrans to produce fatal anaphylactic shock in guinea pigs that were passively sensitized with Type 2 pneumococcal antiserum. Neill and Abrahams (21) and Hehre and Neill (17) showed that some

dextrans gave strong cross-reactions with antibodies evoked by *Salmonella typhi* as well as with a number of other bacterial organisms (cf 20). These various cross-reactions, which occur with both the native and clinical dextrans (17, 18, 20), have proved useful in differentiating and characterizing dextrans from different sources (8, 10, 15, 17).

Nondextran antigens of *Leuconostoc mesenteroides* have been studied in respect to their reactions with leuconostoc antisera (22–24) and in respect to their pneumococcal relationships (19, 25). The nondextran antigens that cross reacted reciprocally with certain pneumococci were distinct from the dextran-pneumococcal cross-reacting system (19). Serologic demonstrations of capsules in some strains of leuconostoc bacteria have been presented (19, 26, 27); these capsules derive their serologic reactivity from nondextran constituents (19, 27, 28). Nondextran antigens are not dependent upon sucrose for their formation but they accompany the dextrans as free, soluble substances in the culture fluids (19) and, hence, may be of significance as trace components in purified dextrans (20).

Stimulated by the observations of allergic reactions in man on infusion of purified dextran preparations in connection with the use of dextran as a plasma expander and by the report that skin reactions to native dextrans occurred in humans (20), Kabat and co-workers (29–35) undertook an extensive series of investigations on the antigenic properties of dextran and other polysaccharides in man. They showed that injection of small amounts of dextran in humans led to formation of precipitating antibodies. The antibodies persisted for several years (34). These findings were confirmed by Maurer (36).

This brief review indicates some of the analogies and some of the differences between the serology of dextran and the pneumococcus polysaccharides. The present study deals with a

TABLE I

Description and physical properties of dextran samples

Lab. No.	Description	N	Ratio 1:6 (Non 1:6 Linkages)	Molecular Weight	
				Light scattering	End group analysis
		%			
	Native Dextrans				
D-7	B-512(F) direct enzymic synthesis[a]			35,400	20,800
D-9	B-512(F) Lm culture[b]	<0.02	17		
D-13	B-742 Lm culture	0.07	2.1		
D-20	B-1374 Lm (Bengers Ltd)		4.3		
D-21	B-1375 Ld[c] (Birmingham strain)		4.2		
D-22	B-1377 Lm (source of Macrodex)		5.2		
D-23	B-1424 Lm culture	0.01	2.5		
W-17	B-1424 Lm (crude)	0.34			
	Hydrolytic Fractions				
D-1	B-512(F)	<0.02		10,700	7,500
D-2	B-512(F)	<0.02		41,600	32,000
D-3	B-512(F)	<0.02		84,400	64,800
D-4	B-512(F)	<0.02		231,000	86,500
D-5	B-512(F)	<0.02		1,350,000	240,000
D-6	B-512(F)	<0.02		8,100,000	
D-26	B-512(F) clinical type	0.01		71,000	
W-1	Pharmacia Macrodex[d] (Lot 76932A)				
W-2	CSC[e] clinical dextran (Lot 84681A)				

[a] Sample D-7 was produced by a method of direct enzymic synthesis that produces dextran of low molecular weight (37).

[b] Produced by *Leuconostoc mesenteroides* culture.

[c] Produced by *Leuconostoc dextranicum* culture.

[d] Swedish clinical dextran manufactured from Strain B-1377.

[e] Commerical Solvents Corporation.

reinvestigation of the antigenic properties of dextran in guinea pigs and presents some quantitative immunochemical studies of the reactions of various native and partially hydrolyzed dextrans with rabbit antisera and in passive anaphylactic transfer studies in guinea pigs.

EXPERIMENTAL

Dextrans. The dextran preparations used in this investigation are described in Table I. The term, native dextran, is used to designate the dextran as synthesized by the microorganism or by direct enzymic synthesis. Native dextrans usually have molecular weights of several million, but Tsuchiya and co-workers (37) have described a method for the enzymic synthesis of low molecular weight dextran. For use as plasma expanders, the native dextrans are partially hydrolyzed and fractionated to appropriate molecular size. Eight native dextran preparations were used in this investigation. The refined native dextrans and the hydrolytic dextran fractions were obtained from the Northern Regional Research Laboratory of the U. S. Department of Agriculture. Characterizations of the native dextrans and the organisms that produced them were described by Jeanes *et al.* (38). With the exception of samples designated by Laboratory Nos. W-1 and W-2, all native and partially degraded dextrans listed in Table I are designated by the NRRL B-number of the causative strain in the Agricultural Research Service Culture Collection at the Northern Regional Research Laboratory (38). Numbers B-1374, B-1375 and B-1377 designate strains which were obtained originally from Benger's Ltd., Dextran Ltd. and Svenska Sockerfabriks AB, respectively, and to which these Culture Collection numbers were assigned. Both strain B-1424 and strain 5 or "A" of J. M. Neill (cf. 8)

originated from ATCC strain 6025 (38) and can be assumed to be fundamentally the same. Strain A has been shown to be a member of an unusual serologic class designated Type A by Hehre (13). The hydrolytic dextran fractions were prepared by Dr. N. N. Hellman by a method of hydrolysis and fractionation similar to that described by Senti and co-workers (39).

Adjuvants. Freund adjuvants (40–42) were prepared in the proportions of 2 ml of saline suspensions of the antigen, 1 ml of melted Falba and 2 ml of mineral oil containing 2.5 mg of killed tubercle bacilli. One-half milliliter of the emulsified antigen was injected into the subcutaneous tissues about 1 cm laterally from the midline on both sides of the neck.

Precipitin studies. The quantitative precipitin determinations were conducted by the method of Heidelberger and Kendall (43) as described by Kabat and Mayer (44). The analyses were carried out by addition of from 0.2 to 2.0 ml of serum, depending upon the potency of the antiserum, to each of a series of tubes containing increasing amounts of dextran in 1 ml of buffered saline. The contents were immediately mixed and stored 1 hr at 37°C and then 5 days at 5°C. The precipitates were centrifuged at 2°C and washed twice with cold saline. It was necessary in most cases to break up the pellet with a stirring rod to insure adequate washing. Nitrogen contents of the precipitates were determined by the micro-Kjeldahl method. The results are recorded on the basis of antibody-N per milliliter of serum.

RESULTS

Anaphylaxis studies

Previous investigators (reviewed in Reference 45) have shown that the pneumococcal polysaccharide will induce immunity in man, mouse, horse, cat and dog, providing that a critical dosage is not exceeded. Neither dextran nor the pneumococcal polysaccharide has been shown to induce antibody formation in guinea pigs unless combined with protein products of the bacterial cell. The following experiments were conducted to determine whether there is a critical dose of dextran that will induce antibody formation in guinea pigs.

A series of 10 guinea pigs of about 300-g body weight were treated with a single intraperitoneal injection of 4-fold serially diluted Macrodex.

The sensitizing dose ranged from 0.00023 to 60 mg of dextran. Four weeks later the animals were challenged by an intravenous injection containing 6 mg of Macrodex. None showed symptoms of shock. These animals were rechallenged 2 weeks later. None reacted.

A second series of 10 guinea pigs were treated with three intraperitoneal injections of the same serial dilutions of Macrodex described above. The first two injections were administered at 2-week intervals and the last injection was made after a further interval of 1 week. These animals were challenged 3 weeks later. Two of the animals exhibited symptoms resembling very slight anaphylaxis, but none showed convincing symptoms of shock.

A third series of 10 guinea pigs were treated as described above with three injections of serially diluted, crude B-1424 native dextran containing 0.34% nitrogen, dry basis. This experiment, likewise, yielded negative results.

Finally, four guinea pigs were treated with large doses of clinical dextran. Two of the animals were treated with Macrodex and two with CSC clinical dextran according to the following schedule: Each animal received four daily subcutaneous injections containing 48 mg of dextran each, followed by 3 days' rest, and then five daily subcutaneous injections. Each animal received a total dose of 432 mg of dextran. One animal from each group was tested by the Schultz-Dale method after 8 weeks. The two remaining animals were tested after 20 weeks. None of the uterine muscles from these animals showed the slightest sensitivity to the homologous dextran.

From these experiments, it does not seem likely that dextran, *per se*, is antigenic in guinea pigs at any dosage level.

Several attempts were made to sensitize guinea pigs to dextran by inoculation with the mucoid phase of B-1424 *Leuconostoc mesenteroides* (Lm) in Freund adjuvants. These attempts were unsuccessful until it was observed that the fluid in which the organisms were suspended contained an appreciable amount of free dextran. Thereafter, the cells were washed three to five times, by centrifugation with fresh saline until the supernatant gave no further test for dextran with a potent rabbit antiserum.

The effect of free dextran on the sensitizing activity of B-1424 Lm was illustrated by the following experiment. A suspension of formalin-killed organisms containing 0.12 mg N/ml (1 ×

10⁹ cells/ml) was washed by centrifugation with saline until the last washing was free of dextran. The original supernatant was estimated by the precipitin test to contain 0.5 to 1 mg of dextran/ml. The washed organisms were resuspended in the original volume of saline and divided into three equal parts. These were recentrifuged. The first part was made up to volume with saline. The second part was diluted to volume with the original supernatant from the organisms. The third part was diluted to volume with a solution containing 0.8 mg of native B-1424 dextran/ml. Oil emulsions of the three suspensions were prepared and each was injected into separate series of four guinea pigs each. Eight weeks later each animal was challenged with 1 mg of dextran. All of the animals in the first group showed symptoms of anaphylaxis and two died in shock. None of the animals in the other two groups showed any symptoms. It was apparent, therefore, that the presence of free dextran in the antigenic suspension inhibited the development of anaphylactic sensitivity to dextran.

Since the dextran in the above experiment was in the oil emulsion with the organisms, and would be continuously released along with the sensitizing antigen, it seemed pertinent to determine whether the same inhibitory effect would occur if the dextran and the antigen were injected separately. Accordingly, 10 guinea pigs were inoculated with oil emulsions of washed B-1424 Lm. Five of the animals then received intravenous injections of serially diluted B-1424 native dextran in doses ranging from 0.1 to 1.6 mg. Ten weeks later all were challenged with dextran. Four control animals died in anaphylactic shock and one showed no symptoms. None of the animals that had been injected with dextran at the time of the sensitizing injection showed any symptoms of anaphylaxis. Thus, relatively small doses of dextran administered at the time the animals received a sensitizing dose of B-1424 Lm inhibited development of sensitivity to dextran.

The experiment recorded in Table II was designed to determine the optimal sensitizing dose of B-1424 Lm when incorporated in 1 ml of adjuvant. Sensitizing doses ranging from 0.001 to 0.32 mg N were injected into eight groups of guinea pigs with five animals on each dosage level. Ten weeks later each animal was challenged with 1 mg of native B-1424 dextran. The

TABLE II

Determination of optimal sensitizing dose of B-1424 Leuconostoc mesenteroides[a]

Sensitizing Dose	No. of Animals	Symptoms of Anaphylaxis		
		None	Non-fatal	Fatal
mg N				
0.001	5	1	4	
0.005	5		1	4
0.01	5			5
0.02	5		1	4
0.04	5	2	2	1
0.08	5	1	1	3
0.16	5	2		3
0.32	5	2	2	1

[a] The incubational period was 10 weeks. The challenging antigen was 1 mg B-1424 native dextran.

TABLE III

Determination of optimal incubational period for B-1424 Leuconostoc mesenteroides in Freund adjuvants[a]

Incubation Period	No. of Animals	Symptoms of Anaphylaxis		
		None	Non-fatal	Fatal
weeks				
2	5	2	2	1
3	5	1	4	
4	5		4	1
6	5		1	4

[a] The sensitizing dose contained 0.01 mg nitrogen. The challenging antigen was 0.5 mg B-1424 native dextran.

results indicate that all dosage levels sensitized some of the animals but the optimal sensitizing dose seemed to be between 0.005 and 0.02 mg N.

Preliminary experiments indicated that the incubational period required for development of sensitivity to dextran after inoculation with B-1424 Lm in oil-emulsion was longer than that usually required with protein antigens. Accordingly, the experiment recorded in Table III was designed to determine the incubational period required to produce maximal sensitivity in guinea pigs. Twenty animals were sensitized on the same day with a dosage level of B-1424 Lm containing 0.01 mg N/ml of adjuvant. Five animals each were challenged after a 2-, 3-, and 4- and

TABLE IV

The antigenicity in guinea pigs of four strains of dextran-producing organisms[a]

Organism and Sensitizing Dose	No. of Animals	Symptoms of Anaphylaxis		
		None	Nonfatal	Fatal
B-512(F)				
0.0025 mg N	5	5		
0.01 mg N	5	5		
0.04 mg N	5	5		
0.16 mg N	5	4	1	
B-1374				
0.01 mg N	5	4	1	1
0.13 mg N	5	4	1	
B-1375				
0.01 mg N	5	2	1	2
0.13 mg N	5	1	4	
B-1377				
0.01 mg N	5	3	1	1
0.05 mg N	5		3	2

[a] The incubational period was 6 weeks. The challenging antigen was 0.5 mg homologous native dextran.

6-weeks incubational period, respectively. The results show that fatal shock may occur in some animals as early as the 2nd week after sensitization, but uniform sensitivity requires an incubational period of 6 weeks.

Table IV records data showing the anaphylactic sensitizing activity of four other strains of dextran-producing organisms. Strain B-512(F)[1] Lm, the organism used for producing clinical dextran in this country, shows very little, if any, sensitizing activity in guinea pigs. This observation was confirmed many times in other experiments not recorded here. Strains B-1374, B-1375, and B-1377 showed some sensitizing activity but none was as potent as Strain B-1424.

The purpose of the experiments recorded in Table V was 3-fold: To determine a) the effect of the time interval between the intravenous injection of dextran and administration of the sensitizing dose of B-1424 Lm; b) the effect of the size of dose of dextran; and c) the effect of heterologous dextran on the inhibition of anaphylactic sensitization in guinea pigs. Groups 1-5 and

[1] The precise designation, B-512(F), indicates a substrain of the original NRRL B-512. The dextrans from B-512 and B-512(F) appear to be identical (38, footnote 27) but differences in the cultures themselves have been reported (28).

TABLE V

Inhibition of anaphylactic sensitization by intravenous administration of native dextran

Group No.	Dextran Injected	Interval between Injections	No. of Animals	Symptoms of Anaphylaxis		
				None	Non-fatal	Fatal
	B-1424					
1	None		8	2	1	5
2	0.1 mg	0	8	5	2	1
3	1.0 mg	0	8	8		
4	1.0 mg	7 days	8	7[a]		
5	1.0 mg	14 days	8	8		
6	None		10	1	1	8
7	1.0 mg	42 days	10	9	1	
	B-512(F)					
8	0.1 mg	0	8	5	3	
9	1.0 mg	0	8[a]	6	1	

[a] One animal died of natural cause during the experimental period. The sensitizing dose of B-1424 Lm (*Leuconostoc mesenteroides*) contained 0.01 mg N. The incubational period was 6 weeks. The challenge dose was 0.5 mg B-1424 native dextran.

groups 8 and 9 were conducted as a single series. The experiment was so arranged that all animals received the same antigenic preparation of B-1424 Lm on the same day. Groups 6 and 7 were treated in the same way but the experiment was conducted at a later date.

The results show that an intravenous dose of 1 mg of B-1424 dextran, when injected concomitantly with the sensitizing antigen, was sufficient to inhibit the development of sensitivity in all eight animals. The 0.1-mg dose was only partially effective. The heterologous dextran, B-512(F), was slightly less effective than the homogologous dextran. The results also show that a 1-mg dose of B-1424 dextran will completely inhibit development of anaphylactic sensitization when the sensitizing dose of B-1424 Lm is administered 1 or 2 weeks after the dextran injection (Groups 4 and 5) and affords almost complete inhibition when the sensitizing injection is delayed as long as 6 weeks (Groups 6 and 7).

The results recorded in Table VI show the effect of molecular size of dextran on the inhibition of sensitivity induced by B-1424 Lm. Groups 1-6, consisting of eight guinea pigs each, were sensitized on the same day with oil emulsified

Inhibition of sensitization by intravenous administration of hydrolyzed B-512(F) dextrans of different molecular weights[a]

Group No.	Dextran Injection	Molecular Weights	Interval between Injections	No. of Animals	Symptoms of Anaphylaxis		
					None	Non-fatal	Fatal
1	None			8		2	6
2	D-1	10,700	0	8	2	1	5
3	D-2	41,600	0	8	4	2	2
4	D-26	71,000	0	8	8		
5	D-4	231,000	0	8	8		
6	D-5	1,350,000	0	8	8		
7	None			10		2	8
8	D-26	71,000	42 days	10	4	3	3

[a] The sensitizing antigen, B-1424 Lm, contained 0.01 mg N. The incubational period was 6 weeks and the challenging antigen was 0.5 mg clinical type B-512(F) dextran.

B-1424 Lm. Groups 2–6 then received intravenous injections consisting of 1 mg of hydrolyzed B-512(F) dextran ranging in molecular size from 10,700 to 1,350,000, respectively. Six weeks later the animals were challenged with 0.5 mg of clinical-type B-512(F) dextran. The degree of inhibition of anaphylactic sensitization afforded by the dextrans ranged from essentially none with dextran of molecular weight (MW) of 10,700 to complete inhibition with dextrans of 71,000 MW and above.

The last experiment (Groups 7 and 8) recorded in Table VI shows that some inhibition is afforded even when the sensitizing injection is administered 6 weeks after the intravenous injection of 1 mg of clinical type dextran.

Precipitin reactions

Antisera for B-1424 and B-1375 were prepared by inoculating rabbits with corresponding strains of formalin-killed and thoroughly washed *Leuconostoc mesenteroides* in mucoid phase. Early sera were prepared according to the schedule of Goodner *et al.* (46) using suspensions of organisms containing 0.13 mg N per ml. Later it was found that higher antibody titers were produced with larger doses of Lm. Accordingly, later sera were prepared by injecting uniform doses of Lm, containing 0.5 mg N, 4 times weekly for 4 weeks and then bleeding according to the foregoing schedule.

In contrast to B-1424, Lm and B-1375 *Leuconostoc dextranicum* (Ld), B-512(F) was a weak antibody producer. However, by increasing both the stimulating dose and the interval of antibody incubation, a precipitating antiserum of unusual characteristics was produced.

The capacity of purified dextrans to react with homologous antiserum and to cross-react with heterologous sera was determined by the quantitative precipitin method (44). The precipitin curves were characterized by a broad plateau showing maximal antibody precipitation in the zone of antigen excess.

Table VII records data for establishing the precipitin curve for B-1424 native dextran (see Fig. 1) with its homologous antiserum (Pool No. 9). Precipitation of maximal antibody nitrogen, 1.45 mg/ml of serum, occurred in the range in which excess antigen and a trace of antibody were found in the supernatants from the precipitates. Maximal antibody precipitation occurred over the range from 1.5 to 3 mg of dextran. The broad range in which both antigen and antibody were found in the supernatants shows that the dextran was serologically heterogeneous.

The data in Table VII were tested for conformity to the two equations,

$$\frac{Ab \ N \ pptd}{D} = a - bD \tag{1}$$

$$\frac{Ab \ N \ pptd}{D} = c - dD^{1/2} \tag{2}$$

introduced by Heidelberger and Kendall (cf. Kabat and Berg (29)) to describe the course of other antigen-antibody reactions by plotting $\frac{Ab \ N \ pptd}{D}$ against the amount of dextran added (D) and against the square root of dextran added. The latter plot gave a straight line conforming to equation 2 between $D = 0.016$ and $D = 0.40$ mg. At $D = 0.48$ to 0.64 mg, however, the plotted points deviated sharply from the straight line, even though these amounts of dextran added were still within the antibody excess zone. Accordingly, equation 2 did not satisfactorily describe the course of the reaction throughout the range of antibody excess. Equation 1 did not conform to any part of the reaction. Kabat and Berg (29) reported that dextran antidextran systems in human sera conform to equation 2. However, their antidextran sera were stimulated

TABLE VII

Precipitin reaction of B-1424 dextran

Antigen Added	Antibody N Precipitated	Ratio Antibody N: Dextran	Tests on Supernatants
mg/ml	mg/ml		
0.016	0.124	7.8	Excess Ab
0.030	0.223	7.4	Excess Ab
0.060	0.396	6.6	Excess Ab
0.090	0.531	5.9	Excess Ab
0.120	0.661	5.5	Excess Ab
0.160	0.805	5.0	Excess Ab
0.20	0.909	4.6	Excess Ab
0.26	0.982	3.8	Excess Ab
0.32	1.03	3.2	Excess Ab
0.40	1.10	2.8	Excess Ab
0.48	1.16	2.4	Excess Ab
0.56	1.21	2.2	Excess Ab
0.64	1.25	2.0	Excess Ab
0.74	1.29	1.7	Excess Ab, trace Ag
0.88	1.33	1.5	Excess Ab, trace Ag
1.00	1.36	1.4	Excess Ab, excess Ag
1.20	1.39	1.2	Trace Ab, excess Ag
1.50	1.45		Trace Ab, excess Ag
2.00	1.45		Trace Ab, excess Ag
3.00	1.46		Trace Ab, excess Ag
4.00	1.32		Trace Ab, excess Ag
5.00	1.14		Excess Ag
6.00	0.764		
8.00	0.453		
10.00	0.439		

Figure 1. Precipitin curves of B-512(F), B-1374, B-1377 and B-1424 native dextrans with B-1424 Lm (*Leuconostoc mesenteroides*) antiserum.

by small injections of dextran and the systems were probably more nearly homogeneous.

Figure 1 compares the precipitin curves of B-1374, B-1377 and B-512(F) native dextrans with B-1424 dextran in B-1424 Lm antiserum. The precipitin curves for the heterologous dextrans followed curves similar to that of the B-1424 dextran, but differed in the maximum antibody precipitated. For example, the B-512(F) dextran precipitated only 70% of the total antibody content. These systems were also serologically heterogeneous as revealed by tests for antigen and antibody in the supernatants.

Precipitin curves were also determined for B-1375, B-512(F) and B-1424 native dextrans in an antiserum produced by B-1375 Ld. The precipitin reactions of B-512(F) and B-1375 dextrans followed about the same course in the antibody-excess zone and both precipitated about the same amount of antibody. The B-1424 dextran precipitated about 90% of the total antibody
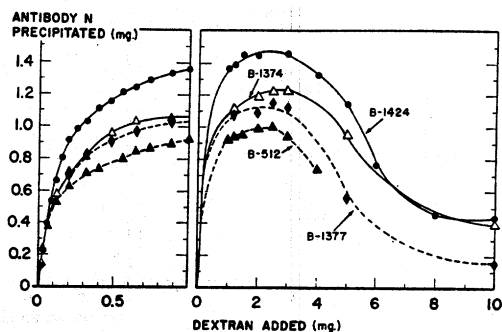
content. None of the three precipitin reactions conformed satisfactorily to equation 1 or to equation 2.

Several attempts to produce antisera to dextran with saline suspensions of B-512(F) Lm were unsuccessful. The possibility that too much of the dextran was removed from the microorganisms by routine washing before use was eliminated as the cause of the failure to produce dextran antibodies. Antigenic potency of B-512(F) Lm was not improved whether the cells were unwashed, or washed 1, 2, 4 or 8 times. Prolonged immunization schedules produced antisera that yielded precipitates with high concentrations of dextran but none or only traces of precipitin in the usual range of maximal precipitation. It thus appeared that intensive immunization with B-512(F) Lm produced antibody to some minor component of purified dextran. Figure 2 illustrates the precipitin reaction of B-512(F) and B-1424 native dextrans with such an antiserum (Pool No. 8). Maximal antibody precipitation for B-1424 dextran was at 10 mg of dextran when 0.055 mg of total nitrogen was precipitated. Forty mg of B-512(F) dextran precipitated 0.063 mg of total nitrogen. It is apparent that B-1424 native dextran contained about four times more of the unidentified minor component than B-512(F) dextran. Other native dextrans studied with antiserum Pool No. 8 included, notably, B-1377 (source of Macrodex) with a peak between 2.5 and 5 mg of dextran and B-742, a highly branched dextran, with a peak at 0.37 mg of dextran. The minor component was also detected in Macrodex and in CSC clinical dextran. Similar quantities of the dextrans gave no precipitates with normal rabbit serum.
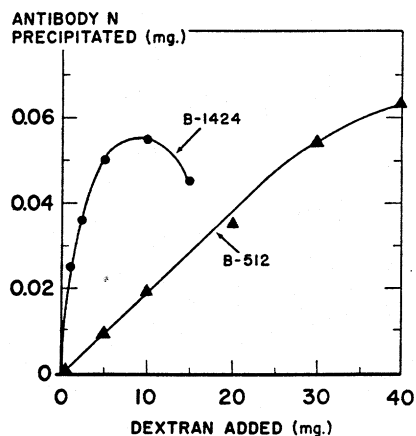
Figure 2. Precipitin curves of B-512(F) and B-1424 native dextrans with B-512(F) Lm (*Leuconostoc mesenteroides*) antiserum.

Figure 3. Precipitin curve of B-512(F) native dextran with B-512(F) Lm (*Leuconostoc mesenteroides*) antiserum showing two widely separated zones of maximal antibody precipitation.

Figure 4. Effect of molecular weight of dextran on precipitating capacity with antiserum.

Immunization schedules that included initial stimulation of antibody formation consisting of five weekly subcutaneous injections of B-512(F) Lm in Freund adjuvants followed by four daily, intravenous injections of cells in saline a week for 3 weeks, yielded antiserum Pool No. 10C shown in Figure 3. The first zone of maximal precipitation represents antibody specific for dextran. The second zone represents antibody for the minor component (or components) of purified dextran.

Antiserum, Pool No. 8, did not transfer passive anaphylactic sensitivity to guinea pigs, probably because the challenging dose (1 mg dextran) did not contain a sufficient amount of the minor component to produce shock. The median anaphylactic sensitizing dose of Pool No. 10C was about 0.17 ml of serum.

Figure 4 records data on the capacity of B-512(F) native dextran, six hydrolytic fractions prepared from it, and one B-512(F) dextran of low molecular weight produced by direct enzymic syntheses, ranging in average molecular weight from 8,100,000 to 10,700, to precipitate antibody from B-1424 Lm antiserum (Pool No. 9). It is evident that as hydrolysis progressed there was a progressive decrease in the amount of antibody precipitated by the lower fractions. The data also indicate that inhibition occurs more readily with an excess of the fractions of smaller molecular weight than with those of higher molecular weight.
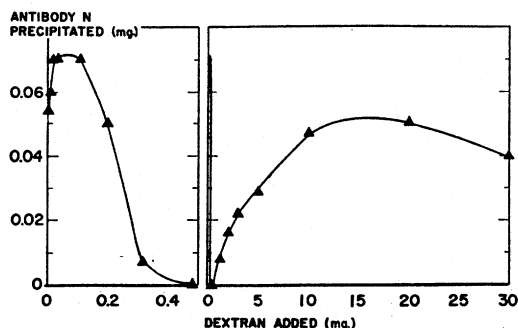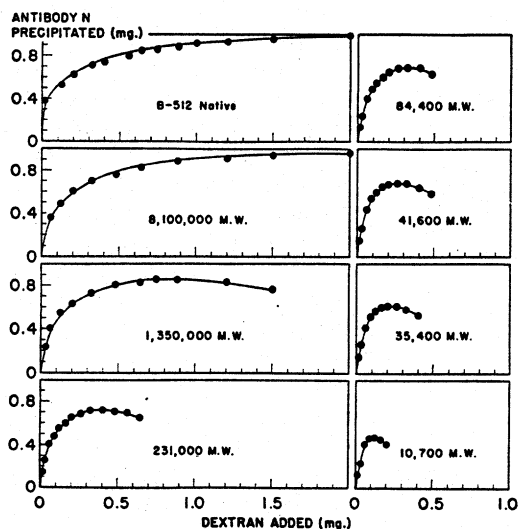
Passive transfer experiments

This section deals with the effect of the molecular size of dextran on its capacity to induce anaphylactic shock in passively sensitized guinea pigs. Seven series of guinea pigs weighing between 280 and 320 g each were passively sensitized by the intraperitoneal route[2] with 116 μg antibody-N of B-1424 Lm antiserum, Pool No. 9. Forty-eight hours later the animals were challenged, intravenously, with different dosage-

[2] It has been shown (48) that the intraperitoneal and intravenous routes of sensitization are equally effective when the animals are challenged after an incubational period of 48 hr.

TABLE VIII
Data showing the mortality ratios of dextran fractions in passively sensitized guinea pigs[a]

Dextran Fraction	Mortality Ratios — Shock dose (in µg)													
	0.85	1.30	2.0	3.0	4.5	6.75	10	15	23	34	51	77	115	173
D-1									0:5	1:5	1:5	2:5	2:5	4:5
D-7	1:5	0:5	1:5	4:5	5:5									
D-2	0:5	2:8	4:8	3:5	5:5	2:2								
D-3		0:1	0:5	1:5	2:5	4:5								
D-4			0:5	0:6	5:6	4:5	3:3							
D-5			0:5	0:5	1:5	5:5								
D-6						0:5	2:5	2:5	4:5	4:5	5:5			
D-9										0:5	1:5	4:5	5:5	

[a] Animals were sensitized with B-1424 Lm (*Leuconostoc mesenteroides*) antiserum containing 116 µg antibody nitrogen.

TABLE IX
Median lethal doses of dextran fractions in passively sensitized guinea pigs

Dextran Fractions	Molecular Weight[a]	LD₅₀
		µg
D-1[b]	10,700	103 ± 27
D-7[c]	35,400	2.2 ± 0.4
D-2	41,600	2.0 ± 0.3
D-3	84,400	4.8 ± 0.7
D-4	231,500	4.3 ± 0.4
D-5	1,350,000	4.9 ± 0.4
D-6	8,100,000	16 ± 3
D-9	(Native)	63 ± 6

[a] Molecular weight by light scattering technique.

[b] Fractions D-1 to D-6, inclusive, were acid degraded products of B-512(F) native dextran.

[c] D-7 was a product of direct enzymic synthesis.

levels of the dextran preparations. Data showing the mortality ratios for the dextran preparations are summarized in Table VIII. From these data dosage-response curves were constructed by the Bliss system of computation (cf. 47). The median lethal doses of dextran were then calculated from the dosage-response curves and are recorded in Table IX. The results showed that the anaphylactic shocking capacity of native dextran was markedly increased by acid degradation. Fractions ranging in molecular weight from 8,100,000 to 35,400 were from 4 to 30 times more effective than native dextran in eliciting anaphylactic shock. However, degradation to a molecular weight of 10,700 substantially reduced the shocking capacity of dextran:

DISCUSSION

The inhibition of sensitization produced in guinea pigs by pretreatment with dextran before sensitization with *Leuconostoc mesenteroides* organisms simulates the "immunological paralysis" discovered by Felton and Ottinger in 1942 (49). The evidence indicates that the paralysis does not persist as long when induced by clinical type dextran as with native dextran. The logical conclusion is that the partially hydrolyzed dextran leaves the system earlier than the native dextran.

The results recorded in this report show that there is a marked difference in the antigenic characteristics of B-512(F) Lm, as compared with B-1424 Lm or B-1375 Ld when these organisms are grown in sucrose-containing media. Pinkes and Neill (27) have also observed differences among strains of *Leuconostoc mesenteroides* with respect to the capacity of their bacterial cells to evoke antibodies reactive with their extracellularly produced dextrans. The findings of others (19, 26–28) offer a possible explanation for these observations. Thus, some strains of leuconostoc bacteria have been shown to be encapsulated (19, 26, 27) and the evidence indicates that dextran is not a component of the capsule (19, 27), except as a contaminant (28). When grown in sucrose-containing media, the

cells of some, but not all, strains carry some dextran on their surfaces or as part of their cellular structure (19, 25) and different strains differ in the ease in which the dextran can be removed by simple washing (28). Possibly a sufficient amount of dextran is firmly held as a contaminant of the capsule or as part of the cellular structure of strain B-1424 leuconostoc to enable the dextran to provide the dominent immunizing complex, whereas, in the B-512(F) strain an insufficient amount of dextran is retained to stimulate antibody formation. It has been reported that traces of dextran remain on B-512(F) cells after washing (28) and demonstrated serologically in this report by the fact that washed B-512(F) cells could stimulate production of dextran antibodies in rabbits when the immunizing dose was incorporated in adjuvants. Such antiserum also contained antibodies against the minor component of dextran. It is probable that the dextran in the washed cells is combined with protein because the pure polysaccharide is not antigenic in rabbits (2, 3).

The precipitin reactions and the passive anaphylaxis experiments with the dextran fractions yielded information on the effect of molecular size of the antigen in immunologic reactions. The native dextran and the hydrolytic fractions above 1,000,000 MW precipitated significantly more antibody than the lower fractions. Fractions ranging from 231,000 to 41,000 MW precipitated from 71 to 68% of the antibody. At 35,400 to 10,700 MW there was a sharp decrease in precipitating capacity. The D-1 fraction precipitated about 47% of the total antibody content. Hence, the minimal size retaining some precipitating capacity is probably somewhat below 10,700 MW. However, since the values given for molecular weights are averages, it is probable that a portion of the precipitate is composed of higher molecular species.

These results are in accord with the reports of Hehre and co-workers (17, 18, 20), who observed a diminished precipitating capacity, in a Type 2 pneumococcus antiserum, with decreased molecular size of hydrolyzed dextran fractions down to and including a molecular weight of about 18,000. They also observed a narrowing both of the range of dextran concentration and of the range of antibody concentrations over which visible precipitation occurred.

Kabat and Bezer (35) investigated the precipitative capacity of a similar series of B-512

dextran fractions in several antidextran human sera. In general, native dextrans precipitated somewhat more antibody than hydrolyzed fractions and their lowest fraction, with a molecular weight of 10,600, precipitated substantially less antibody than did any of the other fractions. But between these extremes (i.e., 35,000–412,000 MW), the capacity of the various fractions to precipitate antidextran did not appear to be correlated with molecular weight. In later studies of these same fractions with a Type 2 antipneumococcal serum, Goodman and Kabat (50) found that this system had more resolving power for detecting differences among the dextran fractions than did the reactions with the human antidextran serum and were more comparable with the results of the present study with antidextran serum.

In agreement with the observations of Kabat and Berg (29), inhibition of precipitation by excess antigen required smaller quantities of the lower fractions than of the higher fractions. Likewise, in the region of excess antibody the slopes of the precipitin curves for the dextran fractions are steeper than that of the native dextran.

In the passive anaphylaxis experiments, maximal shocking capacity was exhibited by dextran preparations ranging from 35,000 to 42,000 in molecular weight and degradation to 10,700 diminished substantially the shocking capacity of dextran. What little activity remained may have resided in the larger molecular species in the fractions. The shocking capacity of fraction D-6 was significantly greater than that of the native dextran. There were no significant differences in the shocking capacities of fractions D-3, D-4 and D-5 which ranged in molecular weight from 84,000 to 1,350,000.

SUMMARY

1. Purified native dextrans, crude native dextran and clinical dextrans did not sensitize guinea pigs actively.

2. Dextran sensitivity could be induced in guinea pigs by inoculation with Leuconostoc mesenteroides NRRL strains B-1374, B-1377, B-1424 and Leuconostoc dextranicum, strain B-1375. However, no sensitivity could be induced with Leuconostoc mesenteroides B-512(F).

3. The sensitivity induced in guinea pigs by Leuconostoc mesenteroides could be inhibited by prior administration of dextran.

4. Antidextran rabbit serum was produced by immunizing rabbits with *Leuconostoc dextranicum* B-1375 and *Leuconostoc mesenteroides* B-1424.

5. Rabbits immunized with *Leuconostoc mesenteroides* B-512(F), without adjuvants, produced antiserum to a minor component of dextran, probably a somatic antigen. Rabbits immunized with B-512(F) with adjuvants produced antibodies both to dextran and to the minor component.

6. The precipitating capacity of dextran with antiserum diminished with deceased molecular size. The lowest fraction with a molecular weight of 10,700 precipitated about one-half of the antibody content of the serum.

7. Hydrolysis increased the shocking capacity of native dextran in passively sensitized guinea pigs. The most effective molecular size was between 41,600 and 35,400.

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